

Candidal Prevalence in Diabetics and its Species Identification

Abstract

Context: *Candida* species is a part of the normal mouth flora. Diabetes mellitus is a predisposing factor for the onset of oral candidiasis. **Aim:** The objective of this study was to estimate salivary glucose in patient with diabetes and healthy individuals, to determine total candidal counts as well as different candidal species in the saliva of patients with diabetes and nondiabetics. **Settings and Design:** A sample size of 80 patients was taken. Out of 80 patients, 30 patients were uncontrolled diabetes patients (Group I), 30 patients were controlled diabetes patients (Group II), and 20 patients were healthy individuals (Group III). **Subjects and Methods:** From all the salivary samples, salivary glucose estimation was done using the glucose oxidase-peroxidase method. Candidal colony-forming units (CFUs) were determined from all the salivary samples. CHROMagar *Candida* medium was used for the identification of various *Candida* species. **Statistical Analysis Used:** One-way ANOVA was used to test for differences between the means of the three groups. Pearson's correlation coefficient test was used to evaluate the relationships between the variables. **Results:** The mean salivary glucose levels were higher in Group I followed by Group II and Group III. The mean candidal CFU was significantly higher in Group I than in Group II and Group III. **Conclusion:** *Candida glabrata* was the predominant species found and more so in patients with diabetes and needs further study. Other common species isolated was *C. albicans*. Species identification can help in better treatment strategies and gain good control over the disease.

Keywords: *Candida* species, candidiasis, CHROMagar *Candida* medium, diabetes mellitus

Introduction

Diabetes mellitus is a predisposing factor to the fungal infections, especially those caused by *Candida* species is well-established.^[1] Patients with diabetes are prone to infection; and oral candidiasis has been found to be more prevalent among patient with diabetes compared with nondiabetic controls.^[2] In the majority, *Candida* is the part of the normal flora and is in the normal state kept under control by means of specific and nonspecific defense mechanisms and by the competition of the microbes in the normal flora.^[3]

It has been reported by several studies that the prevalence of yeast carriage among patients with diabetes could reach up to 54%, and that *Candida albicans* could account for 25%–69% of the isolates.^[2,4] In some studies, the oral carriage rate of *Candida* has been estimated at around 80%.^[2] In diabetes mellitus, the candidal density has also been reported higher than in nondiabetic patients,^[2,5]

not confirmed by other investigators.^[6,7] The disparity in results may be due to the difference in sampling technique.^[8]

Yeasts which are part of the genus *Candida* consist of 150–200 species.^[9] Several species of *Candida* can infect the oral mucosa, *C. albicans* is the most commonly encountered oral fungal agent and because of its greater level of pathogenicity and adherence properties, it may be highly infective. In 40%–65% of healthy adults, *C. albicans* is an oral commensal.^[10] The non-*C. albicans* *Candida* (NCAC) species are a heterogenous group of organisms which are different from each other and from *C. albicans*.^[11] Earlier, *C. albicans* was considered as the only species causing infection, and *Candida parapsilosis*, *Candida tropicalis*, and *Candida guilliermondii* were considered only as occasional pathogens.^[11] The development of new medical therapies for cancer, the increase in invasive medical procedures, the emergence of human immunodeficiency virus and AIDS, and the wide-spread use of broad-spectrum antibiotics lead to the increased recovery of many other

**Shweta Chouhan,
Shreenivas
Kallianpur¹,
Tukaram Prabhu K²,
Manisha Tijare¹,
Sowmya Kasetty¹,
Sandeep Gupta¹**

Department of Oral Pathology, RKDF Dental College and Research Centre, ¹Department of Oral Pathology, People's College of Dental Sciences and Research Centre, ²Department of Microbiology, People's College of Medical Sciences and Research Centre, Bhopal, Madhya Pradesh, India

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Address for correspondence:
Dr. Shweta Chouhan,
Flat No. 1/6, Shakti Nalanda
Tower, New Purulia Road,
Maango, Jamshedpur - 831 012,
Jharkhand, India.
E-mail: shweta_0008@yahoo.
com

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NCAC species causing mucosal infections.^[11] Species such as *C. inconspicua*, *C. lusitaniae*, *C. norvegensis*, and *C. rugosa* have been isolated occasionally from patients.^[12] *C. albicans*, *C. tropicalis*, *Candida krusei*, or *Candida dubliniensis* are the main causative factors.^[3]

This study is aimed at estimating salivary glucose in diabetic and healthy individuals and the determination of total Candidal counts as well as different Candidal species in the saliva of patient with diabetes and nondiabetic individuals. Results obtained in the study might throw light on candidal prevalence in diabetic patients as compared to the healthy ones.

Subjects and Methods

Sample size of 80 patients was taken to conduct the study. The study comprised three groups. Group I consisted of 30 uncontrolled patients with diabetes. They were Type 2 diabetes patients with uncontrolled metabolic state. These patients were not taking any drugs other than those to control diabetes. Group II comprised 30 controlled patients with diabetes. They were Type 2 diabetic patients with their metabolic state under control. These patients were on oral hypoglycemic and not taking any other medication. Group III consisted of 20 healthy nondiabetic ones. These individuals were with no features of diabetes mellitus, and blood glucose levels were within normal limits.

The study was approved by the ethical committee of the People's College of Dental Sciences and Research Centre, Bhopal, with approval number PCDS/Acad/2011-12/1899. The study protocol was explained to patients and the informed consent was obtained from all the patients.

Unstimulated salivary samples were collected from all patients between 8 a.m. and 11 a.m. to avoid circadian variations. Whole saliva was collected by the spitting method for at least 5 min. The salivary samples were transported to the laboratory and were analyzed on the same day. Samples were centrifuged at 3000 rpm for 5–10 min and clear supernatants were processed immediately for estimation of glucose. Glucose was estimated in the supernatant saliva using the glucose oxidase-peroxidase method.^[13-15] A volume of 1000 µL of reagent solution was pipetted into each test tube out of the three test tubes labeled “Blank,” “Standard,” and “Test”. A volume of 10 µL of the standard was added to the test tube labeled as ‘Standard’. A volume of 10 µL of test sample was added to the “Test” of the test tube. These were mixed well and all the test tubes were kept in an incubator at 37°C for 10 min before aspiration. First, the reagent blank was aspirated in the semi-automated analyzer, followed by the standard solution for which the reading was noted, and finally, the test sample was aspirated and the reading was noted. The results were calculated, and the values were expressed as milligrams per deciliter (mg/dL).^[15]

Expectorated saliva was collected in a sterile container. It was immediately concentrated by centrifuging for 10 min.

Supernatant was discarded, and 0.001 µL inoculating loop was used to spread the sample on to Sabouraud's dextrose agar (SDA) plates supplemented with chloramphenicol (10 mg/mL). The loop is held between the thumb and index finger and passed at a 90° angle several times through the initial inoculum into the second quadrant of the plate (streak area 1). The plate is turned 90°, and the process is repeated, streaking into the third quadrant (streak area 2), and finally, after another 90° turn—into the fourth quadrant (streak area 3). The loop is flamed between quadrants unless the inoculum is light or the medium is selective or inhibitory. The plate was incubated overnight at 37°C. After incubation, colony-forming units (CFUs) were counted manually, and the number was multiplied by 1000 and expressed as CFU/mL. CHROMagar *Candida*, a differential and selective medium was used to permit the identification of *C. albicans* and other *Candida* species based on the colony color and characteristics.

Data entry, database management, and all statistical analysis were performed with the SPSS 20.0 trial version (Statistical Package for the Social Sciences, IBM Corporation, Bengaluru) software. One-way ANOVA was used to test for differences between the means of the three groups. Relationships between the variables were evaluated by the Pearson's correlation coefficient. A value of $P < 0.05$ was considered to be statistically significant.

Results

In Group I, out of 30 uncontrolled patients with diabetes, 27 (90%) carried *Candida*, with *Candida glabrata* in 56.6%, *Candida albicans* in 23.3%, and *Candida tropicalis* in 10% of patients. In Group II, 19 persons carried *Candida* in their oral cavity (63.3%). The most common species was *C. glabrata* in 40%, followed by *Candida albicans* in 20%, and *Candida tropicalis* in 3% cases. In Group III, 4 (20%) persons carried *Candida* in their oral cavity with *C. glabrata* as the most common (20%) [Tables 1 and 2].

Salivary glucose levels were significantly higher in diabetic patients than in nondiabetic patients. The mean salivary glucose levels were the highest in Group I (21.93 ± 4.53 mg/dL) followed by Group II (4.73 ± 2.02 mg/dL) and Group III (1.38 ± 0.516 mg/dL). Significant differences ($P < 0.05$) in salivary glucose between all groups were observed.

The mean candidal CFU was significantly higher in Group I (62963 CFU/ml) than in Group II (12,368 CFU/ml)

Table 1: Prevalence of candida among the three groups

Group	Total	Growth, n (%)	No growth, n (%)
Uncontrolled patients with diabetes (Group I)	30	27 (90)	3 (10)
Controlled patients with diabetes (Group II)	30	19 (63.3)	11 (36.6)
Healthy controls (Group III)	20	4 (20)	16 (80)

and Group III (29,000 CFU/ml)]. Comparison of candidal colony count among the three groups was found to be statistically significant ($P < 0.05$) [Figure 1].

In all groups, salivary glucose and candidal colony count were correlated by the Pearson's correlation coefficient test and no statistical significance was found in Group I and II; however, CFU of *Candida* showed a negative correlation with salivary glucose in Group III and was found to be statistically significant ($r = 0.637$; $P = 0.0003$) [Figure 2].

Discussion

In this study, glucose was detected in the saliva of nondiabetic patients, as reported by others.^[16,17] In nondiabetic patients, the mean salivary glucose levels (1.380 ± 0.516 mg/dL) were higher in the present study compared to the other studies, which could be attributed to the carbohydrate-rich dietary pattern of the Indian population.

Salivary glucose levels were significantly higher in diabetic patients (Group I > Group II) than in healthy nondiabetics (Group III). Similar finding has been observed by others,^[17] which suggests that salivary glucose levels likely follow a threshold mechanism. During or soon after birth, *Candida species* colonize mucosal surfaces of human beings and the risk of endogenous infection is ever present.^[18] Patients with compromised host defenses are susceptible to ubiquitous fungi to which healthy people are exposed but are usually resistant.

In various studies, the carriage rate of *Candida* in the oral cavity was different. It could be due to different methods

of sampling. In the oral cavity of diabetic patients, the carriage rate of *Candida* is claimed to be higher. Increased candidal density has been shown to be associated with increased concentration of salivary glucose.^[19] Other investigators have also observed that increased *Candida* reflects increased salivary glucose levels.^[17,19] In the present study, candidal colonies were isolated on SDA [Figure 3]. Colony-forming unit (CFU) is usually recorded, to obtain the clinical data to establish a clinical diagnosis of oral candidiasis.^[19] In this study, the frequency of *Candida* colonization (62.5%) was similar to that observed in other studies (45%–70%).^[20,21]

The present study has confirmed that *Candida* is more prevalent in the oral cavity of patients with diabetes than nondiabetics as observed by earlier workers.^[2,22] Carriage rate was high in patients with diabetes (76.6%) when compared to healthy persons in this study. Carriage rate in patients with diabetes in this study was a little higher than in some studies.^[18,23,24] Our results, in regards to oral carriage rate of *Candida* in healthy individuals was in agreement with other studies.^[18,24] Candidal CFUs were significantly higher in diabetic patients (Group I > Group II) compared with nondiabetic patients (Group III). It was similar to the findings of earlier studies.^[25] *C. albicans* was a frequent species colonizing the mouth as expected [Figure 4]. There was a diversity of other species as well. *C. glabrata* was the most frequent non-*albicans* species (41.25%) [Figure 5]. This was followed by *C. tropicalis*. *Candida albicans* was common in the present study (20%) like in other studies.^[23,24] Kadir *et al.* have found that oral carrier rate and density of

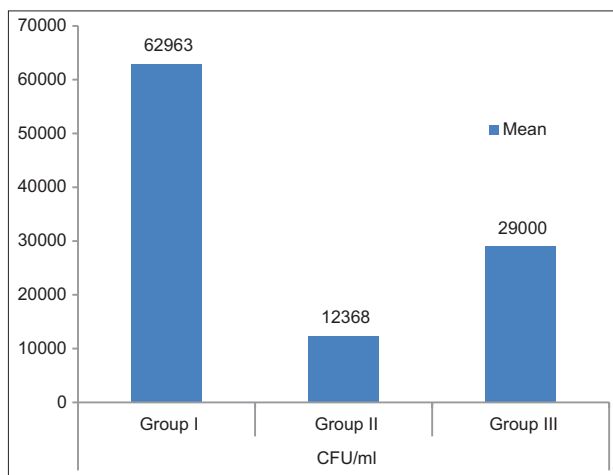


Figure 1: Comparison of colony-forming units of *Candida* among the three groups

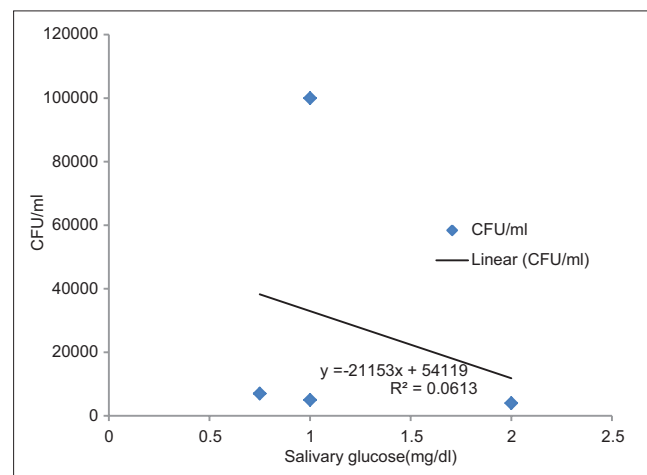


Figure 2: Correlation of colony-forming units of *Candida* and salivary glucose in nondiabetic healthy controls (Group III)

Table 2: Speciation among the three groups by CHROMagar method

Group	Total	Growth	Green (<i>Candida albicans</i>), n (%)	Blue (<i>Candida tropicalis</i>), n (%)	Pink (<i>Candida krusei</i>), n (%)	White/violet (<i>Candida glabrata</i>), n (%)
Group I	30	27	7 (23.3)	3 (10)	0	17 (56.6)
Group II	30	19	6 (20)	1 (3.3)	0	12 (40)
Group III	20	4	0	0	0	4 (20)

Candida species were nonsignificantly higher in the patients with diabetes than in the nondiabetic control patients.^[26]

It has been demonstrated by Epstein *et al.* that carriers and patients with oral candidiasis can be reliably distinguished on the basis of quantitative cultures.^[21] Epstein *et al.* established a correlation between signs and symptoms of candidiasis and high (>400 CFU/mL saliva)-colony counts.^[21] Patients with clinical candidiasis harbor >400 CFU per mL of saliva. Between healthy and patients with diabetes such cutoff limits for CFU may serve as a useful clinical indicator.^[19]

Jafari *et al.* found that *Candida* colonization was more prevalent in the oral cavity of Type 2 patients with diabetes than nondiabetics. The study did not observe any association between salivary glucose level and oral *Candida* colonization in Type 2 patients with diabetes. Higher colonization of *Candida* in diabetics with longer history of diabetes was seen. Results of this study indicated a higher oral *Candida* carriage in Type 2 diabetics and recommended more attention for controlling of

diabetes.^[27] The use of CHROMagar could allow mycology laboratories to rapidly identify *Candida* spp. in clinical samples (Ainscough and Kibbler, 1998). This capability will also enable clinicians to more rapidly make appropriate antifungal choices thus decreasing the patient morbidity and mortality.^[28] Speciation of *Candida* was done based on the color exhibited by the colonies on CHROMagar. Green-colored colonies indicated *C. albicans*, blue-colored colonies indicated *C. tropicalis*, pink-colored colonies indicated *C. krusei*, and white/violet-colored colonies indicated *C. glabrata* [Figure 6]. Within the groups, a significant correlation between salivary glucose and candidal CFUs was present only in nondiabetic patients.

High levels of salivary glucose increase candidal adherence to buccal epithelial cells.^[29] In tissues, salivary glucose forms chemically reversible glycosylation products with proteins during hyperglycemic episodes, and this leads to accumulation of glycosylation products on buccal epithelial cells, which, in turn, may increase the number of available

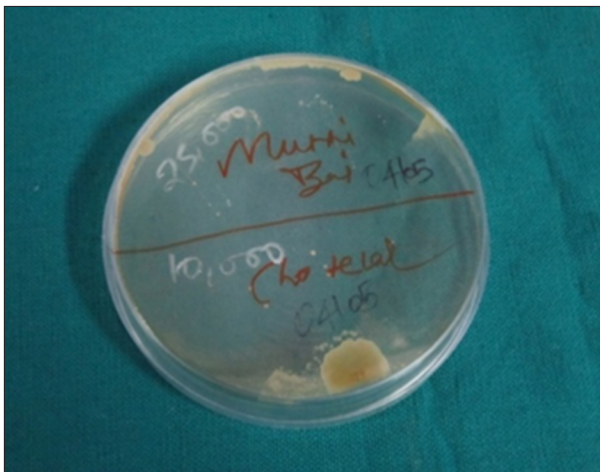


Figure 3: Petri dish showing cream-colored candidal colonies (Sabouraud's dextrose agar)



Figure 4: Petridish showing colonies of different colors: *Candida glabrata* as violet, *Candida albicans* as green (CHROMagar)



Figure 5: Petridish showing confluent growth of *Candida glabrata* (CHROMagar)

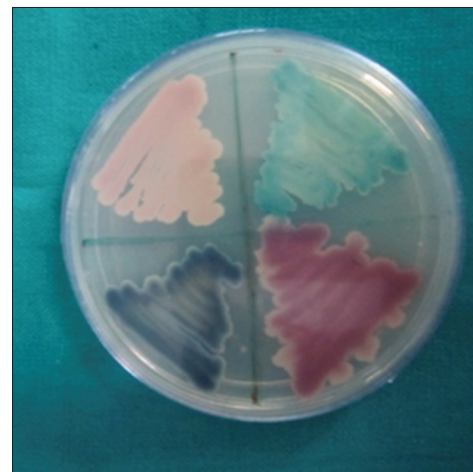


Figure 6: Petridish showing colonies of different colors: *Candida glabrata* as white/violet, *Candida tropicalis* as blue, and *Candida albicans* as green (CHROMagar)

receptors for *Candida*.^[30] Odds *et al.*^[8] and Willis *et al.*^[22] have found an increase in candidal density with increased concentration of salivary glucose. Another consideration is the ability of *Candida* to adhere to the oral epithelium. Adhesion of an organism to the host is a prerequisite for colonization and subsequent infection.^[31] In patients with diabetes, oral epithelium favor adhesion and colonization of *Candida* unlike in nondiabetic patients. It is possible that there may be intrinsic qualitative changes on the cell surface receptors which modulate *Candida* adhesion in diabetic patients.^[32] Other factors that play a role in DM are decreased candidacidal activity of neutrophils, particularly in the presence of glucose.^[33]

Longitudinal studies should be developed to evaluate whether patients with high *Candida* colonization are prone to develop oral candidiasis and which are the clinical factors that predispose them to develop colonization by nonalbicans species, as well as their role in oral disease. In the present study it was found that, in diabetic patients, the increase in salivary glucose levels likely contributes to their increased candidal carriage and the potential for increased susceptibility to oral candidiasis. The findings of the study would be helpful in any further studies which, if done prospectively on a larger cohort, can be confirmatory.

Conclusion

Diabetes Mellitus (DM) is gaining the status of a potential epidemic. Increased oral carriage rate of *Candida* species have been reported in patients with Diabetes Mellitus.

In the present study, we have done review and discussed the clinical data in the literature on the relationship between diabetes and oral candidal carriage and infection.

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Conflicts of interest

There are no conflicts of interest.

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